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Continuous downstream process of monoclonal antibody developed based on the process analysis/understanding and its validation

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Continuous downstream process of monoclonal antibody
developed based on the process analysis/understanding and
the validation
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Manufacturing Technology Association of Biologics(MAB)
Established on September 26, 2013


36 Companies, 5 Universities, 2 National Research Agency, 3 Organizations, (As of April 4, 2022)

http://cho-mab.or.jp/english/
How we developed our CDSP
(our mission or my complicated journey)

Three year project (2018-2020) for continuous manufacturing of mAb

1. Based on our standard platform batch process
   Protein A capture, low pH VI, Polish with 2 columns, VF

2. Process analysis/understanding of each process based on mechanistic models for CDSP

3. Experimental validation (Feed rate = 0.5 to 4 L/d).
   GMP run 10 L/d connected to a perfusion reactor (Goal).
Conversion of batch to continuous

**Batch**
- Capture chromatography step
  - PAC (Bind/Elute operation)
- Buffer exchange + filtration
- Low pH virus inactivation (VI)
- Buffer exchange + filtration
- Polishing chromatography step 1
  - (Bind/Elute or flow-through operation)
- Buffer exchange + filtration
- Polishing chromatography step 2
  - (Bind/Elute or flow-through operation)
- Virus filtration (constant pressure)

**Continuous**
- Multi-column periodic counter-current chromatography (PCCC)
- Continuous buffer exchange + filtration
- Flow-reactor VI
- Continuous buffer exchange + filtration
- Flow-through chromatography 1 (FTC)
- Continuous buffer exchange + filtration
- Flow-through chromatography 2 (FTC)
- Virus filtration (constant flow)

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[1] Continuous capture chromatography
Periodic counter-current operation

Batch operation

Protein A chromatography

Repeated cyclic operation

Periodic counter-current chromatography (PCCC)  2-column PCCC

- Loading to connected 2 columns ($t_{1a}$) and a single column ($t_{1b}$)
- Continuous input, periodic output
- Buffer usage reduction
- 5 to 20 fold concentration

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Mechanistic modeling of capture chromatography process optimization

Productivity = \( \frac{\text{Protein applied}}{(\text{column volume } \times \text{ cycle time})} \)

\[ P = C_0 t_1 F_v / (V_t t_c) = \frac{\text{DBC}}{t_c} \]

\[ \text{DBC} = C_0 V_B / V_t \]

\[ t_c = t_1 + t_2 + t_3 + t_4 + t_5 = t_L + t_{NL} = t_{NL} + a(V_t / F_v) \]

\[ P \text{ increases when } (d \text{DBC}/du) > 1/(d t_c/du) \]

$E^* = a_1^- a_2^- F^*$

$E^* = DBC / SBC$ [-]

$F^* = ud_p^2 / Z D_s = d_p^2 / [D_s (Z/u)]$ [-]

$d_p$: particle diameter
$D_s$: pore diffusivity
$u$: mobile phase velocity
$Z$: bed height

$C_0 = 1\ \text{mg/mL} \quad Z = \text{ca. 5 cm}$

$D_s \times 10^{11} \ [\text{m}^2/\text{s}]$

- MS (85µm): 1.1
- MS Sure (85µm): 1.1
- MS Xtra (75µm): 1.0
- vProA (35µm): 1.0
- vProA (45µm): 1.0
- Prosep UP (60µm): 1.8
- UNO SuprA (57µm): 0.8 Carta

$E^*$ vs. $F^*$

$D_s \times V_t / F_v = Z / u_0$ [min]

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In the real process, the total process volume $V_F$ (concentration $C_F$) and the total process time $t_{tot}$ are fixed. Then, the productivity $P$ is only determined by the bed volume $V_t$.

$$P = \frac{(C_F V_F)}{(t_{tot} V_t)}$$

When we perform multiple runs within $t_{tot} = n_C t_C$, the bed volume $V_t$ decreases, which results in a higher $P$ value. ($n_C$: the number of runs). By increasing $n_C$, $t_C$ decreases. DBC also decreases.

<table>
<thead>
<tr>
<th>$n_C$</th>
<th>Loading $V_1$</th>
<th>non-loading $V_{NL}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multiple cycles results in smaller bed volume. However, due to smaller DBC at short residence time, buffer consumption increases.
Calculation procedure \( V_F(C_F) \) should be processed within \( t_{tot} \).

**Set operating conditions:**
- \( V_F, C_F, t_{tot}, \alpha, RT_{NL}, \Delta p_{max} \)
- \( C_F \): Sample concentration
- \( V_F \): Sample volume
- \( t_{tot} \): Total process time
- \( t_C \): Cycle time
- \( \alpha \): Buffers for non-loading protocol (NL)
- \( RT \): Residence time
- \( RT_{NL} \): Non-loading RT
- \( \Delta p_{max} \): Maximum pressure

**Set resin conditions:**
- \( d_p, SBC, D_s, a_1, a_2 \)
- \( d_p \): Particle diameter
- \( D_s \): Pore diffusivity

**Set residence time RT**

\[
DBC = SBC[a_1-a_2(d_p^2/D_s)/RT]
\]

\[
t_C = (DBC)RT/C_F + \alpha RT_{NL}
\]

\[
n_C = t_{tot}/t_C \quad (n_C: \text{integer})
\]

\[
V_t = (C_FV_F/DBC)/n_C
\]

\[
V_{NL} = \alpha V_t n_C
\]

\[
Z_{max} = \left[ \frac{\Delta p_{max}RTd_p^2}{180\mu} \left( \frac{\varepsilon^3}{(1-\varepsilon)^2} \right) \right]^{1/2}
\]

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Case A: \( V_F = 100 \text{L} \) \( C_F = 1 \text{g/L} \) \( t_{\text{total}} = 12 \text{hr} \) \( V_{\text{buffer}} = 30 \text{ CV per run} \) \( d_p = 85 \mu \text{m SBC} = 55 \text{ g/L} \)

\[
\frac{V_t}{V_{t,\text{ref}}} \quad \text{DBC}
\]

\[
\frac{V_{NL}}{V_{NL, \text{ref}}}
\]

Residence time = \( RT = \frac{V_t}{F_v} \) [min]

\( RT = 1.75 \text{ min} \)
\( V_t = 0.56 \text{L} \) \( n_c = 6 \)
\( V_{\text{buffer}} = 100 \text{L} \)

\( V_t = 2.2 \text{ L} \) \( n_c = 1 \) Buffer volume \( V_{\text{buffer}} = 66 \text{L} \)

\( RT = 7 \text{ min} \), \( RT_{\text{NL}} \) (for non-loading) = 2 min

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**Base case**  
$V_t = 2.1 \text{ L}$  
Buffer volume $V_{\text{buffer}} = 63 \text{ L}$  
Residence time = 7 min  
Residence time = 2 min  
extcept for loading

$V_t = 0.38 \text{ L} \quad n_c = 7$  
$V_{\text{buffer}} = 80 \text{ L}$  
$V_t/F = 1 \text{ min}$
### Table  Calculated $P$ values for Case A, B and C

<table>
<thead>
<tr>
<th>SBC g/L</th>
<th>$d_p$ μm</th>
<th>$n_c$</th>
<th>RT min</th>
<th>$V_t$ L</th>
<th>$V_{NL}^{D)}$ L</th>
<th>DBC g/L</th>
<th>$t_C$ min</th>
<th>$P$ g/(h·L)</th>
<th>$Z_m^{E)}$ cm</th>
<th>$E^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>55$^{A)}$</td>
<td>85</td>
<td>1</td>
<td>7</td>
<td>2.24</td>
<td>67.3</td>
<td>44.6</td>
<td>372.1</td>
<td>7.2</td>
<td>41.6</td>
<td>0.81</td>
</tr>
<tr>
<td>55$^{A)}$</td>
<td>85</td>
<td>6</td>
<td>1.75</td>
<td>0.56</td>
<td>101</td>
<td>29.8</td>
<td>112.2</td>
<td>16</td>
<td>20.8</td>
<td>0.54</td>
</tr>
<tr>
<td>55$^{B)}$</td>
<td>50</td>
<td>7</td>
<td>1</td>
<td>0.38</td>
<td>79.8</td>
<td>37.6</td>
<td>97.6</td>
<td>23.1</td>
<td>9.3</td>
<td>0.68</td>
</tr>
<tr>
<td>90$^{C)}$</td>
<td>50</td>
<td>5</td>
<td>1</td>
<td>0.33</td>
<td>48.8</td>
<td>61.5</td>
<td>121.5</td>
<td>30.4</td>
<td>9.3</td>
<td>0.68</td>
</tr>
<tr>
<td>90$^{C)}$</td>
<td>50</td>
<td>7</td>
<td>0.75</td>
<td>0.26</td>
<td>54.5</td>
<td>55.0</td>
<td>101.3</td>
<td>32.6</td>
<td>8.0</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$^{A)}$ Case A, $^{B)}$ Case B, $^{C)}$ Case C
$^{D)}$ Note that the minimum $V_{NL}$ is given by $\alpha(C_F V_F/SBC)$; 
54.5 L for Case A and B, and 33.3 L for Case C.
$^{E)}$ Bed height due to the pressure limit 0.1 MPa

The most simple case: 3-column PCCC operation

Column 1    Column 2    Column 3

$t^{(1)} = 0$
Wash, Elution

$t^{(1)} = t^{NL}$
CIP Regeneration

$t^{(2)} = 0$
Wash, Elution

$t^{(2)} = t^{NL}$
CIP Regeneration

$t^{(3)} = 0$
2-column loading start

$t^{(3)} = t^{NL}$
2-column loading end

Elution curves

$t_L \geq t^{NL}$

Loading time for the connected 2-columns (tandem column)

Non-loading time (wash, elution, CIP, regeneration)

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The most simple case: 3-column PCCC operation

A Regressive approach to the design of continuous capture process with multi-column chromatography for monoclonal antibodies


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Buffer consumption $V_{NL}^*$ vs. productivity $P$

$V_{NL} = 14$ CV, $V_{NL}^* = V_{NL}/(C_0 \text{DBC})$, $V_{NL,0}^* = V_{NL}/(C_0 \text{SBC}) = 0.204$

- Maximum $P$ for batch and 3C-pccc are similar
- Buffer consumption increases with $P$.
- Buffer consumption for 3C-pccc is smaller than batch by 10-40%.
- When $t_{NL}$ is long, maximum $P$ for 3C-pcc is smaller than batch because of the constraint $t_L \geq t_{NL}$.
- $t_{NL}$ and $V_{NL}$ are important parameters

**Non-loading protocol in this study**

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
<th>RT (min)</th>
<th>time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibrium</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Post load wash</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Wash</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Elution</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>CIP</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{NL}$</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{NL}$</td>
<td></td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>
Summary
- Productivity can be increased both by continuous or repeated batch operation. Namely, the bed volume can be reduced.
- PCCC can reduce the buffer consumption up to 30-40%.
- PCCC process is strongly influenced by the non-loading protocol.
- As PCCC output is not continuous but intermittent, it is not easily connected to the following virus inactivation reactor.
- As PCCC reduces the volume, the volumetric flow rate is reduced for the following processes. This is important to consider for scale-down studies.
- Mechanistic model based analysis is important for the continuous and repeated batch process characterization.
- Optimized repeated batch operation is as efficient as continuous operation.

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[2] Continuous low pH virus inactivation by flow reactor
Tubular reactor or packed bed reactor

Batch
- Capture
- Mixing tank
- Polishing
- Sensor (pH, conductivity)
- Residence time distribution (RTD)

Continuous
- Tubular reactor or packed bed reactor
- pH 3.4 (Low pH)
- Buffer
- pH 7.0 (High pH)
- Polishing
- Sensor (pH, conductivity)
- Residence time distribution (RTD)
[2] Continuous low pH virus inactivation by flow reactor
Tubular reactor or packed bed reactor

1) Collection of PCCC elution curves into a tank
2) Automated pH adjustment for low pH in a stirred tank
3) Incubation for an assured assigned time based on RTD analysis
4) Automated pH and conductivity adjustment for FTC
5) Supply to FTC

- RTD analysis based on mechanistic models for tubular and packed bed reactors.
- Narrow RTD is needed for an efficient reactor.

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Residence time distribution (RTD)

\[ C(t) = C_{\text{max}} \exp \left[ \frac{1}{2\sigma^2}(t/t_R)^2 \right] \]

\[ \sigma^2 = \frac{d_c^2 t_R}{96D_m} \quad (2) \]

Taylor dispersion

Relative RTD

\[ t_R^* = \frac{(t_R - 3\sigma)}{t_R} \quad (3) \]

or

\[ t_R^* = \frac{(t_R - 4\sigma)}{t_R} \quad (3') \]

\[ t_R - 3\sigma = 3600 \, \text{s} \]

\[ t_R^* \Rightarrow 1 \quad \text{narrow RTD} \]
[2] Practical limitations of continuous low pH virus inactivation by flow reactor

Scale-down study is always needed. Let consider the following case.

• Considering the pH electrode/the conductivity cell sizes, the volume of mixing tanks for adjusting pH/conductivity should be 30-50 mL.
• Namely, 60-100 mL + the volume of the reactor is needed.
• PCCC reduces the elution volume by a factor of 5-10. It takes 24 h to accumulate 100 mL PCCC fraction when the feed rate is 1000 mL/d.

\[ C_F = 2 \, \text{g/L} \quad F_1 = 1000 \, \text{mL/d} \quad F_2 = 0.1F_1 = 100 \, \text{mL/d} \]

• It is practically useful to use the automated batch mixing vessel (tank) reactor, which can also work as a **CYCLE SURGE TANK (N-mAb)**.

\[ F_2 = 0.05-0.2F_1 \]

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Bind/Elute chromatography

Target is first bound tightly to the column. Then, it is eluted by changing the mobile phase salt concentration and/or pH.

Flow through chromatography (FTC)

While impurities are bound to the column, the target protein is recovered without adsorption. As the amount of impurities for polish chromatography is small, FTC is a very efficient continuous method.

Choosing the right mobile phase is important.

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[3] Continuous polish  Flow-through chromatography (FTC)

FTC separation mechanism by Ion exchange chromatography (IEC) in terms of distribution coefficient $K$

- While the target molecule is eluted from the column continuously, the impurities are bound tightly.
- The sample loading should be stopped before the breakthrough of the impurities.
- Both the salt concentration (and pH) and the residence time affect the impurity breakthrough.


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How to determine $K$ as a function of $I$ from linear gradient elution (LGE) data

$$GH = I^{(B+1)}/[A(B+1)] \rightarrow K = AI^{-B} + K_C$$

$$GH = g(V_t-V_o) \quad V_t: \text{column vol.}$$
$$\quad V_o: \text{column void vol.}$$

$B$ : the number of binding sites
Flow-through chromatography – model simulation –

1) Linear gradient elution (LGE) experiments
   Prepare $GH-I_R$ plots to determine $A$ & $B$

2) $K = f(I) = AI^{B} + K_C$ for monomer and dimer

3) Single-zone spreading parameter model for elution curves $C/C_0 = f(N, K, V_F)$ where $V_F$ is the sample feed volume.

4) $N$ is calculated by HETP equation, which includes the pore diffusivity, $D_s$ and $K$.

5) BSA monomer-dimer separation on Q Sepharose HP


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**FTC process design method**

![Graph showing elution volume vs. relative concentration](image1)

- Elution volume, $V$
- Relative concentration
- $V_{r}$, $V_{c1}$, $V_{f1}$, $V_{c2}$
- Product, Impurity

**LGE of monomer separation by IEC**

![Graph showing absorbance at 280nm vs. elution volume](image2)

- Absorbance at 280nm
- NaCl concentration
- $I_{R}$, $V_{F}$, $V_{C}$

**Table:**

<table>
<thead>
<tr>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A = 4.0 \times 10^{-5}$</td>
<td>$A = 4.0 \times 10^{-7}$</td>
</tr>
<tr>
<td>$B = 6.5$</td>
<td>$B = 10.8$</td>
</tr>
</tbody>
</table>

**Mobile phase salt concentration, $I_{M}$ [M]**

- $K_{1}$, $K_{2}$, $V_{F}^{*}$, $V_{a1}^{*}$
- 0.15, 0.18, 0.20, 0.25

**Elution volume [mL]**

- FTC process design method
- LGE of monomer separation by IEC


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Table 1  Calculated and experimental breakthrough volumes of monomer and dimer

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K$ [-]</td>
<td>$V_a^*$ [-]</td>
<td>$V_a^*$ [-]</td>
<td>$V_a$ [mL]</td>
<td>$V_a$ [mL]</td>
</tr>
<tr>
<td>monomer</td>
<td>3.5</td>
<td>2.2</td>
<td>3.0</td>
<td>29.0</td>
<td>20</td>
</tr>
<tr>
<td>dimer</td>
<td>44.5</td>
<td>22.7</td>
<td>21.0</td>
<td>216</td>
<td>202</td>
</tr>
</tbody>
</table>

Calculated $K_1=3.8$, $K_2=47$, $248$ mL $=ca.25CV$.

$A_{280}$ [mAU] vs. Elution Volume, $V$ [mL]

Sample 5mg/mL BSA: SIGMA A7511

Conductivity [mS/cm]


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FTC process simulations

Effect of salt concentration, \( I \)
Very sensitive to a small change in \( I \)

Effect of residence time (RT)

\( I = 0.175 \)
\( K_1 = 4.0 \)
\( K_2 = 60.1 \)
\( V_F^* = 24.9 \)
\( V_{a1}^* = 2.3 \)
\( RT = 240 \text{ s} \)

\( I = 0.18 \)
\( K_1 = 3.5 \)
\( K_2 = 44.5 \)
\( V_F^* = 20.0 \)
\( V_{a1}^* = 2.2 \)
\( RT = 120 \text{ s} \)

\( I = 0.18 \)
\( K_1 = 3.5 \)
\( K_2 = 44.5 \)
\( V_F^* = 18.1 \)
\( V_{a1}^* = 2.1 \)
\( RT = 240 \text{ s} \)

\( I = 0.18 \)
\( K_1 = 3.5 \)
\( K_2 = 44.5 \)
\( V_F^* = 20.0 \)
\( V_{a1}^* = 2.2 \)
\( RT = 480 \text{ s} \)

\( I = 0.18 \)
\( K_1 = 3.5 \)
\( K_2 = 44.5 \)
\( V_F^* = 18.1 \)
\( V_{a1}^* = 2.1 \)
\( RT = 480 \text{ s} \)

\( I = 0.18 \)
\( K_1 = 3.5 \)
\( K_2 = 44.5 \)
\( V_F^* = 15.4 \)
\( V_{a1}^* = 1.8 \)
\( RT = 480 \text{ s} \)

\( J.\text{Chem.Eng. Jpn.}, \text{53,} \text{206-213,} \text{214-221(2020)} \)

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Choosing the right mobile phase salt concentration for FTC by ion exchange chromatography (IEC)

- Distribution coefficient $K$ vs. mobile phase salt concentration $I$ curve describes the binding strength.
- Tight binding when $K \gg 100$
- Weak binding and flow through when $K < 10$
- $K$ vs. $I$ curve is important and useful for choosing the right salt concentration $I$.
- Determining $K$ vs. $I$ curve is not easy. A method using Linear gradient elution data is recommended.

- When two different FTC steps are needed, the buffer exchange should be carried out after the 1st FTC.
- Two FTC columns can be connected for eliminating the buffer exchange.
- Searching for the optimum mobile phase is a difficult task.

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[4] CDSP developed at MAB

Capture 2-column PCCC  Low pH virus inactivation  FTC + Virus filtration (VF)

Wash buffer, elution buffer, CIP, buffer
Sample flow rate, $F_1$

- Input
- Impurities
- Output

Batch reaction = 60 min

- pH Low
  - 3.4
  - 1 hr
- pH High
  - 6.5

Sample flow rate $F_2 = 0.05-0.2F_1$

$C_F F_1 = C_E F_2$

Residence time = 20-60 min

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**[4] Concept of CDSP**

**Residence time**
- **PCCC 24h** (30-60 min)
- **VI** (60-80 min)
- **FTC(+VF) 24h** (60 min)

**Capture 2-column PCCC**
- Buffer, Wash buffer, elution buffer, CIP
- Sample flow rate, \( F_1 \)
- Sample flow rate, \( C_F \)
- Impurities

**Low pH virus inactivation**
- pH 3.4
- VI 1hr
- pH 6.5
- Volume= \((24 \times F_1)/a\)
- \(10 < a < 20\)

**FTC + Virus filtration (VF)**
- Sample flow rate \( F_2 = F_1/a \)
- \( C_E \)

**24 hr operation**
- (YMC Contichrom HPLC30)
- Protein A chromatography
- Kaneka or JSR

**Stirred tank reactor**
- Every 24 hs
- The final solution was filtered with 0.2 \( \mu \)m MF.

**24h operation (AKTA Pure 150)**
- JNC; Mixed mode, Cation exchange
- Asahi Kasei Meidal Planova

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Run at MAB GMP facility in Kobe (connected to upstream)
Run at MAB GMP facility (connected to upstream)

Fermentation (perfusion)

Capture (2C-PCC)

Low-pH VI

Polishing+VF(all FT)

KANEKA KanCapA 3G
15 mL x 2
$P_c = 28 \text{ g/(L·h)}$

Tris

Automated single-use reactor

Cellufine MAX IB 100 mL
Cellufine MAX DexS-HbP 100 mL
Planova BioEX 0.001 m²

$F_F = 10 \text{ L/d}$
$C_F = 2 \text{ g/L}$

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[4] CDSP developed at MAB

Capture 2-column PCCC  Low pH virus inactivation  FTC + Virus filtration (VF)

Wash buffer, elution buffer, CIP, buffer
Sample flow rate, \( F_1 \)

\[ C_F F_1 = C_E F_2 \]

Impurities

\[ F_W \]

Residence time = 40-60 min

\[ F_2 = 0.05-0.2 F_1 \]

Sample flow rate \( F_2 \)

Residence time = 20-60 min

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Summary

1. We have developed a continuous downstream process, which consists of 2-column PCCC, a batch VI reactor, and connected 2-column FTC (with VF). The batch VI reactor can work as a buffer or surge tank, and is useful for defining the batch size.

2. Six runs for five day operation were carried out with $C_F=1-3.2$ g/L and $F_F=0.5-3.6$ L/d. GMP run was carried out with $C_F=2$ g/L and $F_F=10$ L/d, which was connected to the perfusion reactor.

3. The recovery was ca. 80%. The monomer purity by SEC was 95%. HCP clearance $<10$ ng/mg-IgG DNA clearance $<1$ pg/mg-IgG.

4. PCCC can reduce the buffer consumption up to 30-40%.

5. As PCCC output is not continuous but intermittent, and reduces the volume, the volumetric flow rate is reduced for the following processes. This is important to consider for scale-down studies, and to connect to the following virus inactivation reactor.

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Summary (continued)

6. Mechanistic model based analysis is important for the continuous process characterization.

7. The process was not optimized. Further improvement of the process efficiency is possible.

8. Scale up to 10-50 L/d is straightforward with the current system.

9. It is also needed to develop the process performance criteria as the productivity itself is not sufficient for the assessment. Even the productivity of the whole process is still difficult to be defined.

10. Buffer consumption of FTC columns was not calculated as the single use was assumed. However, the repeated use with CIP and regeneration may be more environmentally friendly.

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Accelerated method for designing flow-through chromatography of proteins
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Optimization of flow-through chromatography of proteins
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